

# Inefficient purifying selection: the mammalian Y chromosome in the rodent genus *Mus*

Sara A. Sandstedt, Priscilla K. Tucker

Department of Ecology and Evolutionary Biology, and Museum of Zoology, University of Michigan, Ann Arbor, Michigan 48109, USA

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## Abstract

Two related genes with potentially similar functions, one on the Y chromosome and one on the X chromosome, were examined to determine if they evolved differently because of their chromosomal positions. Six hundred fifty-seven base pairs of coding sequence of *Jarid1d* (*Smcy*) on the Y chromosome and *Jarid1c* (*Smcx*) on the X chromosome were sequenced in 13 rodent taxa. An analysis of replacement and silent substitutions, using a counting method designed for samples with small evolutionary distances, showed a significant difference between the two genes. The different patterns of replacement and silent substitutions within *Jarid1d* and *Jarid1c* may be a result of evolutionary mechanisms that are particularly strong on the Y chromosome because of its unique properties. These findings are similar to results of previous studies of Y chromosomal genes in these and other mammalian taxa, suggesting that genes on the mammalian Y evolve in a chromosome-specific manner.

## Introduction

Chromosomal sex determination has evolved separately in some plants, fishes, birds, insects, and in mammals (reviewed by Bull 1983). Interestingly, the properties of Y chromosomes are similar in some of the independently evolved instances that have been studied (e.g., Fridolfsson et al. 1998; Steinemann and Steinemann 1998; Tucker et al. 2003; Cepitis and Ellegren 2004; Zhang 2004). One model of sex

chromosome evolution, derived mainly from studies in *Drosophila*, is that chromosomal sex determination may evolve from genic sex determination by the gradual accumulation of sex-specific genes in the region of the sex-determining gene, where they would be more likely to be inherited by offspring of the correct sex. Suppression of recombination may keep these genes that are beneficial to males from being inherited by females but also allow divergence and erosion of the sex-specific genes (reviewed by Rice 1996).

While much is known about the evolution of the Y in *Drosophila*, less is known about other organisms. For example, the dioecious plant *Silene latifolia* appears to differ from *Drosophila* and mammals because its Y chromosome may not have experienced degeneration and its X lacks dosage compensation (reviewed by Negrutiu et al. 2001). Because independently evolved Y chromosomes may behave differently from the model, it is useful to study the evolution of the Y chromosome in depth in other organisms, including mammals.

Distinguishable X and Y chromosomes that evolved from a homologous chromosome pair are found in all mammalian groups including eutherians (placental mammals), monotremes, and marsupials (reviewed by Toder et al. 2000). Sex chromosomes have characteristics that may cause their genes to evolve with different rates and patterns from genes on other chromosomes. Two of these characteristics are that most of the Y is completely genetically linked and it has a small effective population size, each of which may interfere with natural selection through several mechanisms (recently reviewed by Rice 1996; Charlesworth and Charlesworth 2000; Lahn et al. 2001; Tucker et al. 2003). We are interested in determining if genes on the Y chromosome show patterns of evolution consistent with an interference with natural selection, which causes purifying selection to be inefficient, or if these genes

\*Present address: Department of Epidemiology, University of Michigan, 109 Observatory, Rm 2010 School of Public Health Building I, Ann Arbor, MI 48109, USA  
 Correspondence to: Sara A. Sandstedt; E-mail: sandsted@umich.edu

evolve in a locus-specific manner related to their particular functions.

If features of the Y chromosome interfere with purifying selection, the ratio of nonsynonymous changes per nonsynonymous site to synonymous changes per synonymous site (dN/dS) should be less than one, but more than the baseline dN/dS ratio caused by purifying selection. The gene under study may be compared to a similar gene that is thought to undergo purifying selection. Some Y chromosomal genes have "gametologs" which are related genes found on the X chromosome, with similarity retained from before recombination between the X and Y chromosomes ceased (Garcia-Moreno and Mindell 2000). If purifying selection is interfered with by the properties of the Y, the dN/dS of a Y chromosomal gene is expected to be higher than the dN/dS of its X chromosomal gametolog.

Recently, pairwise comparisons of gametologs were made between human and mouse, and human and macaque (Wyckoff et al. 2002). Indeed, the dN/dS of the Y chromosomal genes was higher than the dN/dS of their X chromosomal gametologs. It was concluded that either the Y chromosome genes are continuing to evolve new functions or that a Y chromosomal effect is causing more nonsynonymous substitutions. These results are intriguing, but it is also valuable to examine this question in a finer-scale phylogenetic context in which less time has elapsed and fewer multiple hits have occurred. We chose to examine gametologous gene pairs within the mouse genus *Mus*. One advantage of using this particular system is that two Y chromosomal genes (*Sry* and *Zfy*) have already been examined in these species. However, each of these genes may have evolved as it did because of factors unrelated to their positions on the Y chromosome (Tucker and Lundrigan 1993; Jansa et al. 2003; Tucker et al. 2003), compelling further study.

Here we examine gametologs that are not as likely to be affected by complicating factors. We sequenced and analyzed *Jarid1d* from the Y chromosome [formerly *Smcy*, Selected mouse cDNA on Y (Agulnik et al. 1994a)] and *Jarid1c* from the X chromosome [formerly *Smcx* (Agulnik et al. 1994b)] in species of the mouse genus *Mus* (family Muridae, subfamily Murinae). These genes are members of the highly conserved Jarid subfamily (reviewed by Patsialou et al. 2005) and were renamed to reflect that relationship in January 2004 (<http://www.informatics.jax.org>). *Jarid1c* partially escapes X inactivation in mice (Wu et al. 1994b; Sheardown et al. 1996) and in humans (Wu et al. 1994a). *Jarid1c* also appears to escape X inactivation in other primates, horses, dogs, whales, and elephants (Jegalian and Page 1998), al-

though more *Jarid1c* mRNA is expressed in female mouse brains than *Jarid1c* and *Jarid1d* combined in male mouse brains (Xu et al. 2002). In gametolog pairs in which the X-linked gene escapes inactivation, males may be constrained to have exactly one functionally equivalent gene on the Y chromosome to maintain an equivalent gene dosage (reviewed by Lahn and Page 1997; Disteché 1999). *Jarid1d* and *Jarid1c* were chosen for this study because the genes are similar in expression patterns (Agulnik et al. 1994a) and seem likely to share functions (Agulnik et al. 1999; but see Jensen et al. 2005). Thus, differences between them are more likely to be due to their chromosomal positions than differences between other gene pairs that have very different expression patterns or functions. We report a Y chromosomal effect on the evolution of *Jarid1d* when compared to *Jarid1c* in the mammalian genus *Mus*.

### Materials and methods

**Species.** Thirteen taxa, including some of the most commonly studied species of the genus *Mus*, were used (see Lundrigan et al. 2002 for collecting information). The genera *Mus*, *Mastomys*, and *Hylomyscus*, are members of the rodent family Muridae and the subfamily Murinae, the Old-world Mice and Rats. Many of the same species used in the study of rodent *Sry* (Tucker and Lundrigan 1993) were used here to make the results more comparable. The sister group to *Mus* is unknown, although there is evidence from DNA hybridization and nuclear DNA sequence for a sister group relationship with the *Praomys* group (Catzefflis and Denys 1992; Chevret, et al. 1994; Jansa and Weksler 2004). Members of the *Praomys* group (*Hylomyscus alleni* and *Mastomys hildebrandtii*) were used as outgroups in rooted trees in this study.

**Amplification of the target sequences.** In mice, *Jarid1d* and *Jarid1c* share the same genomic structure of 26 exons (Agulnik et al. 1999). *Jarid1d* is present in marsupials, indicating that *Jarid1c* and *Jarid1d* diverged at least 120 million years ago (Agulnik et al. 1994a). *Jarid1d* encodes several of the H-Y antigens that can cause the rejection of male-donor tissue transplants in female recipients (Scott et al. 1995; Wang et al. 1995). Both *Jarid1d* and *Jarid1c* are members of the jumonji family of transcription factors (Takeuchi et al. 1995) and *Jarid1c* mutations are related to X-linked mental retardation in humans (Jensen et al. 2005).

The polymerase chain reaction (PCR) (Saiki et al. 1985) was used as described, with primers developed

specifically for these species (Sandstedt and Tucker 2004), to amplify a fragment that includes exons 23 and 24, a portion of exon 25, and introns 23, 24 and 25 from both genes in all taxa. All sequences were deposited in GenBank (accession No. #AY260478-AY260503).

### Analysis

**Phylogeny.** *Jarid1d* and *Jarid1c* nucleotide sequences were aligned using Clustal X (Thompson et al. 1997) and adjusted by hand using amino acid translations as a guide with the program MacClade 4.0 (Maddison and Maddison 2000). Unambiguous alignments of exon sequences were obtained for each gene separately using Clustal X (Thompson et al. 1997). Although there were 687 bp in this region of *Jarid1d* and only 660 in *Jarid1c*, their comparison required the same length sequence for both genes. To produce equally long sequences, X and Y sequences were aligned together using Clustal X. These alignments were adjusted by hand using a conceptual translation in MacClade as a guide. Characters that aligned with gaps were removed, totaling 30 bases from *Jarid1d* and 3 from *Jarid1c*. Maximum parsimony as implemented in PAUP\* v4.0b10 (Swofford 2002) was used to build unrooted gene trees from the *Jarid1c* and *Jarid1d* nucleotide sequences. The branch-and-bound option was used. Characters were equally weighted and unordered. *Jarid1c* and *Jarid1d* intron and exon data were also combined with sequences from four other nuclear genes in parsimony and maximum likelihood analyses as described (Tucker et al. 2005).

**Silent and replacement substitutions.** Because of the small number of substitutions in the sequences studied, a method that is particularly appropriate for small samples was used (Zhang et al. 1997). For this analysis, the uncorrected numbers of replacement and silent substitutions were counted along the branches of the most parsimonious nuclear tree, with its root removed. Ancestral sequences were reconstructed using the program Anc-Gene (Zhang and Nei 1997; Zhang et al. 1998; Zhang 2004). This program first determines the ancestral amino acid sequences using a distance-based Bayesian method, and then reconstructs the nucleotide sequence at each node. Both the Poisson model and the JTT empirical model of amino acid substitution under the extended parsimony mode of Anc-Gene were used. The resulting ancestral sequences were compared using the Nei and Gojobori method (Nei and Gojobori 1986) implemented in MEGA 2.1 (Kumar et al. 2001). Replacement and silent changes were mapped along each branch on the nuclear tree

topology, separately for *Jarid1c* and *Jarid1d*. The numbers of replacement and silent changes per branch for each gene were added together, then compared using Fisher's exact test, which is appropriate for small samples, implemented in the Fisher program (Zhang et al. 1997). Similar analyses were done with parsimony ancestral reconstructions using the ACCTRAN and DELTRAN methods of ancestral reconstruction in PAUP\* v4.0b10 (Swofford 2002).

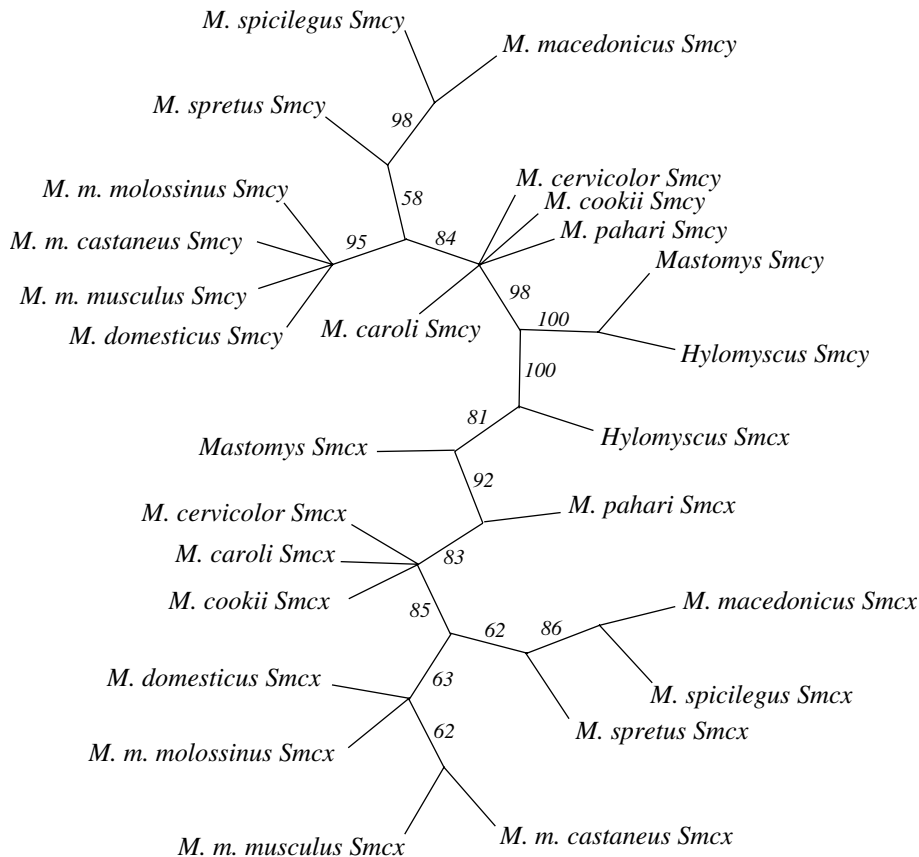
The number of potential sites for a replacement change, N, and the number of potential sites for a silent change, S, were calculated using the Nei and Gojobori method (Nei and Gojobori 1986) implemented in MEGA 2.1 (Kumar et al. 2001). N and S were each averaged over the branches of the *Jarid1d* and *Jarid1c* trees, separately. The dN/dS of each gene was calculated using the changes counted using Anc-gene and the averages of N and S.

### Results

**Phylogenetic analyses.** Eight unrooted equally most parsimonious trees were recovered from the branch-and-bound search of 26 OTUs (13 taxa, *Jarid1d* and *Jarid1c* of each). As expected, two distinct clades, one made up of *Jarid1d* genes and one made up of *Jarid1c* genes, show that these two loci have evolved separately since before the origins of the species examined here (Fig. 1). This topology also suggests that if gene conversion has occurred, it has not included this portion of these genes.

The tree topology built using only *Jarid1d* and *Jarid1c* is unresolved in several clades. To obtain a well-supported and completely resolved topology for use in ancestral reconstruction, maximum parsimony and maximum likelihood outgroup-rooted trees were built using sequences from six nuclear genes including *Jarid1d* and *Jarid1c* (Tucker et al. 2005). These trees are identical to each other, but this topology differs from the *Jarid1d*- and *Jarid1c*-specific tree from the first analysis in two ways. The branches that were previously unresolved are now resolved, and the relationship of *Hylomyscus* and *Mastomys* is changed (Fig. 2). This tree topology was converted to an unrooted topology for the analysis.

**Ancestral reconstruction and counting changes.** Different methods of ancestral reconstruction using the nuclear gene tree topology (Fig. 2) led to different results. ACCTRAN, DELTRAN, and Bayesian-based reconstructions differ in a few sites at most nodes. The results of both types of Bayesian reconstructions, Poisson and JTT, are the



**Fig. 1.** *Jarid1d/Jarid1c* maximum parsimony majority rule consensus tree for members of the genus *Mus* and members of a putative sister group, *Hylomyscus alleni* and *Mastomys hildebrandtii*. Numbers are bootstrap values.

same, and each node has a posterior probability of  $\geq 0.99$  for protein reconstruction  $\times$  DNA reconstruction.

Numbers of replacement and silent substitutions within the genus *Mus* were counted on each reconstructed tree. Using the ACCTRAN reconstructions, *Jarid1c* has 5 replacement and 14 silent changes. *Jarid1d* has 22 replacement and 17 silent changes (Table 1). The exact numbers of substitutions differ with the different methods of reconstructing ancestral sequences but in each case the results are similar: There are more replacement substitutions relative to silent substitutions in *Jarid1d* than in *Jarid1c*. For each reconstruction method, Fisher's exact test shows a significant difference in the numbers of synonymous and nonsynonymous substitutions between *Jarid1d* and *Jarid1c* ( $p < 0.05$ ) (Table 1). When replacement and silent substitutions of the most distantly related taxa, *Hylomyscus* and *Mastomys*, are included with those of *Mus*, the difference is significant only when the DELTRAN reconstructions are used. This disparity in results can be traced to the long branch between the *Hylomyscus/Mastomys* clade and the *Mus* clade, where uncorrected distances may not be appropriate measures.

## Discussion

Higher-than-average dN/dS are found in the mammalian sex-determining gene *Sry* (Tucker and Lundrigan 1993; Whitfield et al. 1993; reviewed by O'Neill and O'Neill 1999; Jansa et al. 2003), and in *Zfy* (Tucker et al. 2003). The dN/dS of *Sry* and *Zfy* are also higher than the dN/dS of their X chromosomal gametologs, *Sox3* and *Zfx* (Pamilo and Waugh O'Neill 1997; Wyckoff et al. 2002; Tucker et al. 2003) (Table 2). The results of previous research on *Sry* and *Zfy* are compatible with either a Y chromosomal effect on the efficiency of purifying selection or alternate explanations invoking locus-specific effects such as adaptive evolution or relaxed functional constraints (Tucker and Lundrigan 1993; Whitfield et al. 1993; Hurst 1994a, b; Lee et al. 1995; Tucker et al. 2003). If unique properties of the Y chromosome are responsible for the patterns of evolution in *Sry* and *Zfy*, a similar pattern should be seen in other Y chromosomal genes, including *Jarid1d*.

However, it is possible that the relatively high dN/dS of *Sry* and *Zfy* is a result of natural selection on gene functions that have changed compared with their counterparts on the X chromosome. Generally,

**Table 1. Comparison of replacement and silent substitutions found in *Jarid1d* and *Jarid1c***

Ancestral reconstruction method	<i>Jarid1d</i>					<i>Jarid1c</i>					Fisher's exact test ( $P$ ) <sup>f</sup>
	$n^a$	$N^b$	$s^c$	$S^d$	$dN/dS$	$n$	$N$	$s$	$S$	$dN/dS$	
ACCTRAN	22	505.36	17	151.36	0.366	5	505.64	14	151.36	0.126	0.03
DELTRAN	22	505.64	19	151.36	0.347	16.5	485.84	41.5	141.16	0.132	0.01
Poisson and JTT <sup>c</sup>	89	505.45	66	151.39	0.404	26.7	485.66	43.25	171.34	0.283	0.01

<sup>a</sup>Number of replacement substitutions counted in *Mus* clade.

<sup>b</sup>Average potential replacement substitutions per branch in *Mus* clade.

<sup>c</sup>Number of silent substitutions counted in *Mus* clade.

<sup>d</sup>Average potential silent substitutions per branch in *Mus* clade.

<sup>e</sup>Poisson and JTT Bayesian reconstruction results were the same.

<sup>f</sup>Test of significance of difference in number of nonsynonymous ( $Jarid1d_n$ ,  $Jarid1c_n$ ) and synonymous ( $Jarid1d_s$ ,  $Jarid1c_s$ ) substitutions.

genes expressed in many tissues (such as *Zfx*) have a lower dN/dS than genes expressed in few tissues (such as *Zfy*), and genes expressed in the brain (such as *Sox3*) have significantly lower dN than genes expressed in other tissues (such as *Sry*) (Duret and Mouchiroud 2000). Thus, *Zfy* and *Sry* may have relatively high dN/dS because of positive selection for new functions or because of their limited patterns of expression instead of their locations on the Y chromosome. *Jarid1c* and *Jarid1d* were chosen for this study because the difference in their dN/dS is less likely to be affected by gene function (Agulnik et al. 1999; but see Jensen et al. 2005) or expression patterns (Agulnik et al. 1994a, b).

Neither *Jarid1c* nor *Jarid1d* has diverged rapidly. Along some branches of the *Mus* tree (Fig. 2), there are no substitutions in either gene. Although this did not substantially affect our conclusions, it does indicate that tests designed for less-diverged sequences are preferable. One such test (Zhang et al. 1997) showed that *Jarid1d* has significantly more replacement substitutions than *Jarid1c* has. This result is consistent with a three-taxon study of *Jarid1c/d* in human, mouse, and horse, in which the median dN/dS was 0.17 for *Jarid1d* and 0.02 for *Jarid1c* (Agulnik et al. 1997). Though the exact numbers reported by Agulnik et al. (1997) differ from those that we find using other methods, this indicates that there are relatively more replacement substitutions in *Jarid1d* in both closely related and distantly related taxa.

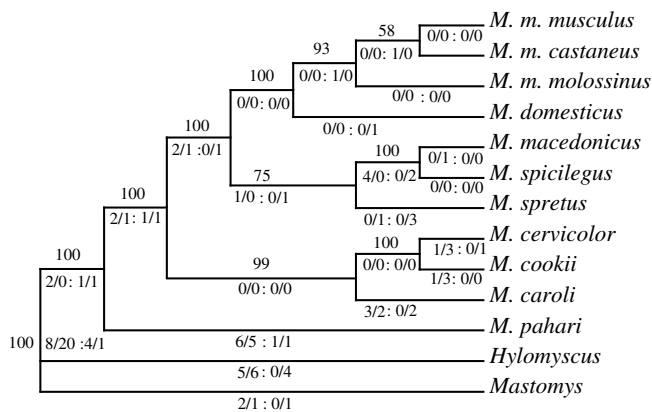
Although the dN/dS of *Jarid1d* on the Y chromosome is higher than the dN/dS of *Jarid1c* on the X chromosome (0.40 and 0.28, respectively, when calculated using the Poisson method), they are similar relative to other X-Y gene pairs. This is not necessarily what would be expected based on the length of time that *Jarid1d* and *Jarid1c* have been diverging from one another. The mammalian X and Y likely diverged from each other in a series of steps, separated by time. The present-day signatures of these steps are strata, defined by the synonymous distance between pairs of gametologs on the X and Y (Lahn and Page 1999). The gene pairs *Sox3/Sry*, *Zfx/y*, and *Jarid1c/d* diverged at different times as shown by the X chromosomal strata of the X chromosomal members of the pairs (Lahn and Page 1999; Sandstedt and Tucker 2004). Since *Jarid1c* is either in X chromosomal stratum 1 or 2, it has been diverging from *Jarid1d* longer than *Zfx* [dN/dS = 0.04 (Tucker et al. 2003)], a stratum 3 gene, has been diverging from *Zfy* [dN/dS = 0.38 (Tucker et al. 2003)]. The relatively subtle difference between *Jarid1d*'s and *Jarid1c*'s dN/dS may be due to greater functional constraints on *Jarid1d* than on other Y chromosomal genes or to lesser functional constraint on *Jarid1c* than on other X chromosomal genes (in the part of the gene studied) (Table 2). Data from Wyckoff et al. (2002) also show that the difference in dN/dS between *Jarid1d* and *Jarid1c* is smaller than the difference between several other X-Y gene pairs in pairwise comparisons of mouse genes with human genes.

**Table 2. Comparison of dN/dS of gametologous gene pairs in the genus *Mus***

Gene	Chromosome	$dN/dS^a$	Source
<i>Jarid1d</i>	Y	0.404	This paper
<i>Jarid1c</i>	X	0.283	This paper
<i>Sry</i>	Y	0.478	(Jansa et al. 2003)
<i>Sox3</i>	X	0.088 <sup>b</sup>	(Pamilo and Waugh O'Neill 1997)
<i>Zfy</i>	Y	0.38	(Tucker et al. 2003)
<i>Zfx</i>	X	0.04	(Tucker et al. 2003)

<sup>a</sup>Calculated using various methods.

<sup>b</sup>From human-mouse comparison.



**Fig. 2.** Single most parsimonious tree using *Jarid1d*, *Jarid1c*, *Sry*, *B2m*, *Zp-3*, and *Tcp-1* (Tucker et al. 2005) for the genus *Mus*. Rooted with outgroups *Hylomyscus alleni* and *Mastomys hildebrandtii*. Numbers above the branches are bootstrap values. Numbers below the branches are  $Jarid1d_n/Jarid1d_s$ ;  $Jarid1c_n/Jarid1c_s$  reconstructed using the ACCTRAN method.

Another implication of the position of these gene pairs in strata, which diverged at different times, is that the elevated dN/dS of Y chromosomal genes cannot be completely attributed to positive selection for new functions. If Y chromosomal genes gained new functions soon after they stopped recombining with their X chromosomal gametologs, they would have many nonsynonymous changes during that time, but few later after their new functions were obtained (Wyckoff et al. 2002). That pattern has not been seen in mouse X-Y gene pairs from strata 1 through 3; instead a high nonsynonymous substitution rate continues along recent branches within the genus *Mus*, not just in ancestral portions of the mammalian tree (current study; Tucker and Lundrigan 1993; Tucker et al. 2003).

Sequence from the autosomal locus *Jarid1b* from *Mus musculus* corresponding to the sequenced region of *Jarid1c* and *Jarid1d* is available in GenBank (accession No. BC048180). The sequence of *Mus musculus Jarid1a* was not alignable with this region. About 120 amino acids could be aligned with confidence between the *Jarid1b/c/d* genes. There are no nonsynonymous changes in *Jarid1c* in this region and five nonsynonymous changes in *Jarid1d*. No nonsynonymous substitutions of *Jarid1d* change amino acids that are conserved in both *Jarid1b* and *Jarid1c*. This may indicate that *Jarid1d* remains evolutionarily constrained by specific, important codons, perhaps because of a need for dosage compensation relative to *Jarid1c*.

Genes that survive on the Y chromosome either have acquired critical male-specific functions or are required for equivalent dosage relative to their X-

linked gametologs. *Jarid1d* is functionally constrained and ubiquitously expressed, while its X-linked gametolog *Jarid1c* escapes X inactivation to some degree in many tissues. We find that there are more nonsynonymous changes in *Jarid1d* than in *Jarid1c*, but it is a subtle difference relative to other X/Y gene pairs. Together these factors suggest that dosage requirements have had a greater impact on the evolution of *Jarid1d* than male-specific functions.

While it is possible that non-Y-specific effects may be responsible for the pattern of evolution of *Jarid1d*, the results of this study, combined with the results of studies of other Y-linked genes in these same taxa (Tucker and Lundrigan 1993; Jansa et al. 2003; Tucker et al. 2003), strongly suggest that there is a Y chromosomal effect on gene evolution in the genus *Mus*. The Y chromosomal effect occurs for genes with different expression patterns, found in one or many copies. The X chromosomal gametologs of these genes differ in their X inactivation patterns and their strata. Taken together, these observations suggest that (1) a higher dN/dS is a constant feature of Y chromosomal gene evolution, which is not limited to a burst of nonsynonymous change soon after X-Y gene pair divergence and (2) it occurs in dissimilar genes. The results reported herein were obtained from gametologs assumed to be functionally equivalent, using closely related taxa, and in a well-supported phylogenetic context. Indeed, they are in accord with and strengthen the results of a pairwise comparison of six X-Y gametologs in distantly related mammals in which the dN/dS was always higher in the Y chromosomal gene (Wyckoff et al. 2002).

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